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PATENT
Attorney Docket No. SYP-116

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT(S):	Fuchs et al.		
SERIAL NO.:	08/726,093	GROUP NO.:	1631
FILING DATE:	October 4, 1996	EXAMINER:	Marschel, A.
TITLE:	METHODS AND KITS FOR HYBRIDIZATION ANALYSIS USING PEPTIDE NUCLEIC ACID PROBES		

CERTIFICATE OF FIRST CLASS MAILING UNDER 37 C.F.R. 1.8

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Beverly Horvitz

Assistant Commissioner for Patents
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1. Transmittal Form (1 page)
2. Amendment and Reply (5 pages)
3. Request for Extension of Time (1 page)
4. Fee Transmittal (1 page)
5. Check in the amount of $380.00
6. Copy of Provisional Application No.: 60/019282 (30 pages)
7. Return Receipt Postcard

blundell709/6.1054928-1

FEE TRANSMITTAL

Note: Effective October 1, 1997. Patent fees are subject to annual revision

AUG 22 2000 U.S. PATENT & TRADEMARK OFFICE

	Complete if Known
Application Serial Number	08/726,093
Filing Date	October 4, 1996
First Named Inventor	Fuchs
Group Art Unit	1631
Examiner Name	Marschel, A
Attorney Docket No.	SYP-116

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310	Design filing fee	
150	Provisional filing fee	

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Independent Claims		- 3 =	x $ 78.00 =	
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FEE CALCULATION (continued)

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50	25	Surcharge – late provisional filing fee or	
130	130	Non-English specification	
2,520	2,520	For filing a request for reexamination	
110	55	Extension for reply within first month	
380	190	**Extension for reply within second month**	**380.00**
870	435	Extension for reply within third month	
1,360	680	Extension for reply within fourth month	
1,850	925	Extension for reply within fifth month	
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300	150	Filing a brief in support of an appeal	
260	130	Request for oral hearing	
130	130	Petitions to the Commissioner	
50	50	Petitions related to provisional applications	
	240	Submission of Information Disclosure Statement (37 CFR 1.97(c))	
130	130	Submission of Information Disclosure Statement (37 CFR 1.97(d))	
760	380	Filing a submission after final rejection	
760	380	For each additional invention to be examined (37 CFR 1.129(b))	
		Other (Specify)	

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SUBTOTAL (1) 0

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SUBTOTAL (3) 380.00

TOTAL **($) 380.00**

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Respectfully submitted,

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TRANSMITTAL FORM

IPE JC9 AUG 22 2000 PATENT & TRADEMARK OFFICE

Application Serial Number	08/726,093
Filing Date	October 4, 1996
First Named Inventor	Fuchs
Group Art Unit	1631
Examiner Name	Marschel, A.
Attorney Docket No.	SYP-116

ENCLOSURES *(check all that apply)*

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PROVISIONAL APPLICATION NUMBER 60/019282

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SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
60/019,282 PROVISIONAL	06/07/96				

APPLICANTS

LEON W.M.M. TERSTAPPEN, HUNTINGDON VALL, PA.

CONTINUING DATA********
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FOREIGN/PCT APPLICATIONS********
VERIFIED

FOREIGN FILING LICENSE GRANTED 06/29/96 ***** SMALL ENTITY *****

Foreign priority claimed / 35 USC 119 conditions met / Verified and Acknowledged	Examiner's initials	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
☐ yes ☐ no / ☐ yes ☐ no		→	PA	2			$75.00	

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TITLE

APPARATUS AND METHOD FOR CELL ANALYSIS

Form PTO-1625
(Rev 5/95

(FACE)

PATENT APPLICATION

APPROVED FOR LICENSE ☐

INITIALS

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CONTENTS

1. Application papers
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POSITION		ID NO.	DATE
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PATENT APPLICATION SERIAL NO. 60/019282

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214 [illegible]

PTO-1556
(5/87)

BAR CODE LABEL

U.S. PATENT APPLICATION

SERIAL NUMBER	FILING DATE	CLASS	GROUP ART UNIT
60/019,282 PROVISIONAL	06/07/96		

APPLICANT

LEON W.M.M. TERSTAPPEN, HUNTINGDON VALL, PA.

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STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS	FILING FEE RECEIVED	ATTORNEY DOCKET NO.
PA	2			$75.00	

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TITLE

APPARATUS AND METHOD FOR CELL ANALYSIS

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60/019282

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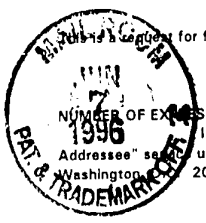

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INVENTOR(S)/APPLICANT(S)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
TERSTAPPEN	LEON	W.M.M.	Huntingdon Valley, Pennsylvania PA

1-00

TITLE OF THE INVENTION (200 characters max)

APPARATUS AND METHOD FOR CELL ANALYSIS

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STATE PA ZIP CODE 19103-2307 COUNTRY USA

ENCLOSED APPLICATION PARTS *(check all that apply)*

X Specification *Number of Pages* 15 2 Small Entity Statements

X Claims *Number of Claims* 5

X Drawing(s) *Number of Sheets* 2 X Other (specify) Abstract

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

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The Invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

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Respectfully submitted

DANN, DORFMAN, HERRELL AND SKILLMAN
A Professional Corporation
Attorney for Applicant(s)

By Patrick J. Hagan
Patrick J. Hagan

☐ Additional inventors are being named on separately numbered sheets attached hereto

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ABSTRACT

Apparatus and methods are disclosed for quantitatively analyzing the particulate content of a test sample such as blood comprising particles of a target substance of interest present in a fluid carrier medium. The apparatus comprises a receiver having at least one side which is optically transparent and, in a preferred embodiment, includes parallel ferromagnetic lines. The test sample is exposed to one or more labelling materials such as bioactive colloidal magnetic particles, fluorescently labelled antibodies, fluorescent probes and the like, either outside or in the receiver. After the target particles are appropriately labelled, the receiver is placed in a magnetic field. The target particles, such as cells associated with the bioactive colloidal magnetic particles, will become immobilized in a linear array on both sides of each ferromagnetic line. Optionally the unbound material can be removed from the receiver. Analysis of the particles is performed by a mechanical tracking system by which the optical system, the chamber or a combination of both moves along both sides of all ferromagnetic lines in the receiver. Detection of the particles is performed by illumination of a spot along the ferromagnetic line concurrently with or followed by the detection of the scattered light and emitted light of the particles. Discrimination between different types of particles aligned along the ferromagnetic lines is obtained by differences in the scattered light and in the emission spectrum of the light.

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APPARATUS AND METHOD FOR CELL ANALYSIS

by Leon WMM Terstappen, MD, Ph.D.

TECHNICAL FIELD OF THE INVENTION

This invention relates to the differentiation and enumeration of biological particles in bodily fluids such as blood, urine and bone marrow.

BACKGROUND OF THE INVENTION

Analysis of the cellular composition of bodily fluids is used in the diagnosis of a variety of diseases. Microscopic examination of cells smeared or deposited on slides and stained by Romanowsky or cytochemical means has been the traditional method for cell analysis. Introduction of impedance based cell counters in the late fifties has led to a major advance in the accuracy of cell enumeration and cell differentiation. Since then various other technologies have been introduced for cell enumeration and differentiation such as Fluorescence Activated Flowcytometry, Quantitative Buffy Coat Analysis, Volumetric Capillary Cytometry, Laser Scanning Cytometry and various image analysis systems. Fluorescence based flowcytometry has made the most impact in the current ability to discern different cell types in heterogeneous cell mixtures. Simultaneous assessment of multiple parameters of individual cells which pass the measurement orifice at a speed of 1,000 to 10,000 cells indeed is a powerful technology. Their are however limitations of the technology such as the inability to deal with high cell concentrations (blood needs to be diluted), impractically to detect infrequent cells and the inability to reexamine the cells of interest. To overcome these limitations samples to be analyzed are typically subjected to various enrichments schema's such as erythrocyte lysis, density separations,

immunospecific selection or depletion of cell populations.

SUMMARY OF THE INVENTION

The present invention provides apparatus and methods for quantitatively analyzing the particulate content of a test sample comprising particles of a target substance present in a fluid carrier, which particles have selectable response characteristics to impingement thereon of focused rays of radiant energy in the optical wave band. The method of the invention comprises the steps of providing a receiver having a wall portion that permits transmission of radiant energy, the receiver including capture means for immobilizing the target particles in a linear array, and the capture means being operable to receive and immobilize particles from a zone in the receiver having a predetermined volume; introducing a quantity of said test sample into the receiver for a time sufficient to allow the target particles to be immobilized on said capture means in a substantially linear array corresponding in thickness to about the size of the particles; directing focused rays of radiant energy in the optical range along the linear array of particles; and detecting the response of the target particles within said zone to the impingement of radiant energy thereon, as a measure of the quantity of target particles in the test sample.

The apparatus of the invention comprises a receiver having the above-described characteristics, means for directing focused rays of radiant energy in the optical range along the linear array of particles and means for detecting the particle response to the impingement of radiant energy on such particles, so as to allow differentiation and enumeration of the particulate content of the test sample. In practice,

particle quantitation can be carried out by a mechanical tracking system, an optical illumination / detection system and a signal processing / read out system.

In a preferred embodiment, a wall portion of the receiver has a surface with the capture means superimposed thereon and the detecting step comprises detecting the particle response over an area of the wall surface including at least a portion of the linear array of particles, as a measure of the proportion of the detected particles in the above-mentioned predetermined volume.

According to a particularly preferred embodiment, the apparatus and method are utilized for the quantitation of bioparticles magnetically labelled with colloidal magnetic particles. In this embodiment, the capture means comprises a magnetic line which is magnetized to generate a magnetic field gradient within the receiver along the magnetic line, so as to cause immobilization of the magnetically labelled bioparticles on the magnetic line.

Alternatively, the method of the invention may be used for the quantitation of non-magnetic bioparticles, which comprise or are adapted to comprise at least one binding site, with the capture means comprising a line of binding agent having specific binding affinity for the binding site(s) of the bioparticles and the suspension in the receiver is subjected to conditions causing binding of the bioparticles to the binding agent.

The present invention provides a unique particle analysis apparatus and method which obviates the need for a complicated fluidic system such as used in flowcytometry, as well as the need for random searches for target particles such as used in image analysis and the need to analyze large numbers of

non-target particles such as used in most traditional cell analysis techniques. The invention thus relates to an apparatus and method which enables a rapid determination of the cell composition of a blood specimen or other bodily fluid.

The present invention is especially adapted for the differentiation and enumeration of bioparticles in bodily fluids. For example, peripheral blood can be incubated with probes, such as the colloidal magnetic particles which are the subject of U.S. Patent 4,795,698 (Owen et al.), prepared such as to have specific binding affinity for certain bioparticles of interest which enable the linear orientation of such bioparticles in the apparatus of the invention. The blood sample is also incubated with a probe which enables the identification of particle types by means such as fluorescent nucleic acid stains or fluorescently labelled monoclonal antibodies. This incubation can take place inside or outside the receiver of the apparatus, which in a preferred embodiment comprises parallel ferromagnetic lines disposed on a surface of a transparent wall portion thereof. After appropriate incubation time, the apparatus is placed in a magnetic field of which the magnetic field lines are directed perpendicular to the ferromagnetic lines in the receiver (North-South configuration). The magnetic gradient will orient the magnetically labelled bioparticles along both sides of each ferromagnetic line. To prevent multiple particles in the vertical plane the thickness of edges of the ferromagnetic lines should be of controlled thickness, preferably less than twice the size of the target particle. Enumeration and discrimination between the different types of particles aligned along the ferromagnetic lines is achieved by an optical detection system which measures differential

physicochemical properties of the particles, such as light scatter, absorption and fluorescence.

DESCRIPTION OF THE DRAWINGS

Figure 1 is a set of photographs taken with an inverted fluorescent microscope from whole blood in which the leukocytes are aligned along the ferromagnetic line. The blood was stained with Acridine Orange and magnetically labeled with a CD45 antibody.

Figure 1A shows a ferromagnetic mesh with a width of 20 μm, a thickness of 5 μm and spaces of 63 μm between the lines. Leukocytes are visible as white circles along the ferromagnetic lines. No leukocytes are present along the 48 μm wide perpendicular ferromagnetic line. The photograph was taken with an inverted fluorescent microscope at a 100X magnification and using simultaneously transmitted as well as blue excitation.

Figure 1B shows is substantially the same image as Figure 1A except that only blue fluorescent light was used. As a result, the ferromagnetic lines are no longer visible and only the aligned leukocytes are visible.

Figure 2 shows an apparatus embodying the present invention including a receiver which permits the analysis of subpopulations of particles.

Figure 2A is a plane view and Figure 2B is a perspective view of a schematic representation of an apparatus of the invention.

DETAILED DESCRIPTION OF THE INVENTION

In a preferred embodiment of the invention, as noted above, bioparticles are effectively quantitated in an apparatus 2, as illustrated in

Figure 2, with the bioparticles bound to at least one colloidal magnetic particle, for capture on a plurality of magnetic lines 3 which are magnetizable, e.g., via magnets 4a and 4b to generate a magnetic field gradient within the receiver 5 along the magnetic lines, the magnetic field gradient being of sufficient strength to achieve immobilization. Preferably, the magnetic lines are spaced substantially equidistant from one another along the length thereof, so as to constitute parallel lines, with the space 6 between such parallel lines being at least twice the average diameter of the target particles. In this particular embodiment, magnets 4a and 4b generate magnetic flux lines directed substantially transversely to the parallel magnetic lines 3.

As shown in Figure 2, the magnetic lines are disposed on the surface of a transparent wall portion 7 of the receiver. The magnetic line may be continuous or discontinuous.

Alternatively, non-magnetic bioparticles comprising or being adapted to comprise at least one binding site may be analyzed according to this invention using an apparatus in which the capture means is a line of receptor material having specific binding affinity for the binding site(s) of the bioparticles.

If desired, the magnetic lines may be arranged in a grid including a plurality of parallel intersecting lines disposed parallel to the magnetic flux lines, whereby the intersecting lines do not generate a magnetic field gradient therealong as can be seen in Figure 1.

The technique for immobilization of magnetic target particles described herein is an improvement on those described in U.S. Patent 5,200,084 and published

international application WO 94/11078, the entire disclosures of which are incorporated by reference in the present specification as though set forth herein in full.

The important parameters of the receiver of the apparatus are the width, height and shape of the linear capture means, the height of the receiver and the optical transparency of the material at least at the side adjacent to the capture means. Preferably, lines of ferromagnetic material are superimposed on the optically transparent surface of the receiver which prevents interference of obstacles in the optical detection path and further aids the alignment of the particles in the vertical plain. Optimal shapes of the ferromagnetic lines and their position with respect to the magnets is taught in U.S. Patent Application No. 08/424,271, the entire disclosure of which is incorporated by reference in the present specification, as though set forth herein in full. The total length of the ferromagnetic lines determines the particle capacity of the chamber. For example, in the receiver shown in Figure 2, a total length of the ferromagnetic lines of 150 mm will have a total track length of 300 mm. When a ferromagnetic wire is superimposed on the surface of the receiver to serve as the capture means, the height of the wire above the surface should be adequate to provide a capture strip for the target particles on each side of the magnetic wire. Preferably, the height of the magnetic wire above the receiver wall surface is no greater than the average diameter of the target particles. Since two sides of the line are available for particle immobilization, an average particle size of 10 μm will thus result in a particle capacity of 30,000. With a ferromagnetic line height of 5 μm, a line width of 20 μm and 80 μm spaces between the lines this can be

achieved in a chamber with, for instance, a 5 mm length and 3 mm width. The chamber can be configured to hold any predetermined volume of test sample in which case the number of target analyses along the ferromagnetic lines is proportional to the target analyte number per volume unit of bodily fluid. For example, a chamber height of 0.13 mm results in a chamber volume of $\pm$ 2mm^3 (2 μl) and could for instance hold this volume of whole blood the particle capacity is then sufficient for all leukocytes present in this volume of blood.

In another embodiment of the invention a known volume of bodily fluid is passed through the chamber while in the magnetic field and the number of target particles along the line is proportional to the number of target particles present in the bodily fluid volume passed through the chamber.

A primary advantage of the apparatus and method of the invention is that only the target population of interest is positioned in the analysis zone and the particle capacity is therefore not restricted by the total number of particles present.

Analysis of the target particles is accomplished by directing focused rays of radiant energy in the optical range along the linear array of immobilized particles, and detecting the selected response of the particles to the impingement of the radiant energy thereon. As used herein, the expression "radiant energy in the optical wave band" includes at least the ultra-violet, visible and infra-red wavelengths of the electromagnetic spectrum.

One of the essential components of a flow cytometer is its hydrodynamic focusing fluidic system which provides the mean to align all cells present in a cell mixture in a horizontal plane and pass them by a measurement orifice at velocities of 2-5 m /sec.

The invention described herein provides a similar alignment, but of target particles only. In differentiating and enumerating the target particles the difference between the present invention and traditional flowcytometry is that in practicing the present invention either the optical system is moved along the ferromagnetic lines, the ferromagnetic lines are passed by the optical system or a combination of both movements may be employed, both in separate directions of the horizontal plane.

The analytical system of this invention can be quite similar to that used in flow cytometry and optical analysis of particles fixed in linear areas as described by de Grooth B G, et al, in Cytometry 1985, 6:226-33. The light source preferentially a laser is focused on a small spot, generally < 20 μm, the specifications of the shape and dimensions of the spot is large determined by the sensitivity which is required for the measurement as taught by Shapiro and Doornbos. H. Shapiro, Practical Flowcytometry, Alan R. Liss, 1995; R. Doornbos et al., Cytometry, 15: 267-271 (1994); R. Doornbos et al., Cytometry, 14: 589-594 (1993). The choice of the light source(s) is largely dependent on the application in which it will be used. The use of detection optics similar to those used in CD players, solid state lasers and fluorescent dye's excitable by these lasers, such as nucleic acid stains TO-PO3 and LDS-751 and fluorescent probes CY5 and APC conjugated to monoclonal antibodies or other target identifiers such as single chain antibodies is included within the scope of the invention.

The following examples further describe in some detail manners of using aspects of the present invention and set forth the best mode contemplated by the inventor for carrying out the invention, but are not be construed as limiting the invention.

Example I

Leukocyte Differentiation in Whole Blood

A blood sample is incubated with a ferrofluid labeled pan leukocyte antibody (CD45) and a fluorescent nucleic acid dye. The incubation can take place in or outside the receiver. The receiver is then introduced into a magnetic field and the leukocytes align on both sides of the ferromagnetic lines. Figure 1 shows the alignment of leukocytes in 1 μl of blood. In the example shown in the figure, to 1 μl of blood 0.4 μg of a 130nm CD45 labeled ferrofluid, 3 ng of Acridine Orange and 10 ng of Ethidium Bromide was added and incubated for 10 minutes. The sample was then placed into a receiver of which the bottom consist of a microscope coverslip on top of which a nickel mesh was placed. The mesh consisted of ferromagnetic lines composed of nickel with a width of 20 μm and thickness of 5 μm, spaces of 63 μm were between the lines. The mesh used also contained 48 μm ferromagnetic lines positioned perpendicular to the other lines. Magnets were placed on both sides of the receiver, the orientation of the field lines (--> H) and the position of the North (N) and South (S) pole of the magnets are indicated in the figure. Cells exhibiting the antigen recognized by the CD45 ferrofluid are attracted to the highest magnetic gradient which is present at the edges of the lines positioned perpendicular to the magnetic field lines. Acridine Orange is taken up by the nucleated cells and when excited with blue light (460-500nm) the dye taken up by the nucleus will emit green light whereas dye taken up by the intracytoplasmatic granules of granulocytes will emit red light. Ethidium Bromide is taken up only by cells of which the cell membrane is not intact (dead cells) can be excited by blue light (480 - 580nm) and emits deep red

light. The photograph shown in Figure 1 was taken after placing the chamber in an inverted fluorescent microscope with blue excitation permitting the visualization of the emitted fluorescence in addition to regular light which permit the visualization of the ferromagnetic lines. In Figure 1, the cells which emit fluorescence light can easily be distinguished from the rest of the blood components and are aligned only besides those lines placed perpendicular to magnetic field lines. Discrimination between the cell types is achieved by the detection of selected emission spectra and lightscatter properties of the cell types. It is obvious for those skilled in the art that probes with various specificity's can be used to align different target population and probes with various specificity's and different fluorescence excitation and emission can be used to differentiate between the aligned targets. Also one or more excitation wavelength can be used to discriminate between the targets.

Example II

Immunophenotypic differentiation in whole blood

A blood sample is incubated with a fluorescent nucleic acid dye and ferrofluid labeled with an antibody directed against a cell surface epitope such as for instance CD4 expressed on T-helper lymphocytes and monocytes or CD34 present on progenitor cells. The incubation can take place in or outside the receiver. The receiver is then introduced into a magnetic field and the cells exhibiting the cell surface antigen recognized by the bioactive ferrofluid align on both sides of the ferromagnetic lines. Although all nucleated cells are fluorescently labeled only those which are adjacent to the ferromagnetic lines are target cells and will be

identified as such by the optical detection system. When the target cell frequency is, however, low as is the case for progenitor cells in peripheral blood identified by CD34 in normal donors (1-10 CD34+ cells/ill) the likelihood increases that non-target cells present by coincidence along the ferromagnetic lines influence the accuracy of the enumeration. The likelihood that non-target cells are present by coincidence along the ferromagnetic line and thus mistakenly enumerated as a target cell can be reduced by decreasing the total length of the ferromagnetic line. This can for instance be achieved by decreasing the number of ferromagnetic lines in the chamber.

An alternative approach is not to use a fluorescent nucleic dye but to use a fluorescent labeled monoclonal antibody or other probes directed against the same cell type as the bioactive ferrofluid. Preferably this probe is directed against a different epitope as the bioactive ferrofluid. In this approach only the target cells are fluorescently labeled and identified as such. A drawback of the latter procedure is, however, that it requires a higher sensitivity of the detection system. Other approaches include those generally used in flowcytometry and generally referred to as multi-color and or multidimensional analysis. In this case a bioactive ferrofluid is used to align the particles of interest along the ferromagnetic lines and a variety of monoclonal antibodies or other antigen specific probes labeled with different fluorochromes are used to identify different characteristics or subsets within the immunomagnetically immobilized particles.

Example III

Identification and Examination

of Infrequent Cell Types

With decreasing frequency of a target population it becomes increasingly more difficult to reliably detect, enumerate and examine the target population. Not only is there an increasing demand on the specificity of the identifiers, i.e. probes or a combination of probes, the need arises for a specific target enrichment technique in addition to the need to process larger volumes of the bodily fluid. Table 1 below illustrates this by showing the frequencies of various cell populations among the nucleated cells in peripheral blood of normal individuals.

TABLE 1

Cell Frequency	Cell Number	Targets Cells
1:1/1:10	10,000-1,000/μl	granulocytes, lymphocytes
1:10/1:10^2	1,000-100/μl	monocytes, eosinophils
1:10^2/1:10^3	100-10/μl	basophils
1:10^3/1:10^4	10-1/μl	CD34+ cells
1:10^4/1:10^5	1,000-100/ml	CD34+, CD38- cells
1:10^5/1:10^6	100-10/ml	tumor cells
1:10^6/1:10^7	10-1/ml	tumor cells
1:10^7/1:10^8	1,000-100/l	tumor cells

For the analysis of infrequent cells such as CD34+ cells or a subset thereof or in case of disease potential circulating tumor cells the amount of blood needed to reliably detect, enumerate and examine the target population needs to be substantially larger then 1 μl. Practical implications for the analysis of larger blood volumes are a substantially longer processing time

for example for the flow cytometric analysis of a 1 ml blood sample the erythrocytes in the sample are typically lysed which is accompanied with a 10 fold dilution of the sample. Typical sample flow rates in a flow cytometer are 1 μl/sec, the 10 ml volume will thus be analyzed after 2.78 hours. The need for enrichment of the target population and an increase in its concentration is thus clearly desired. A variety of enrichment methods are commercially available such as the utilization of magnetics (Miltenyi Biotec GmbH, Dynal) or affinity columns (Cell Pro). Success of these procedures is determined by carry over of non targets, recovery of targets, the ability to concentrate the target and the ability to analyze the target after the procedure. Introduction into a receiver of a sample from a bodily fluid of which targets are concentrated and non targets are reduced permits the enumeration and examination of the target population. For example to a vessel containing 10 ml of blood a bioactive ferrofluid is added which identifies cells of epithelial origin, after appropriate incubation the vessel is placed in an open field high gradient magnetic separator as taught by Liberti et al. in U.S. Patent 5,186,827 and after separation the blood components not magnetically attached to the wall of the vessel are removed. The cells separated can now be resuspended into a smaller volume when the vessel is placed outside the magnetic field, to this volume fluorescent labeled probes can be added and after incubation the sample is again placed in a high gradient magnetic separator. After separation the supernatant including excess reagents are removed and the separated cells are resuspended in a volume which fits in the chamber. Assuming the 10 ml of blood contained 10^8 cells, a carry over of 0.01% of the cells would result in 10^4 cells which is within the range of the cell capacity of the example of the receiver of the

apparatus of this invention. Given a target cell frequency of 1 in 10^7 and a recovery of 70%, 7 target cells would be among these cells which is sufficient for identification and further characterization if the detection probes and optical detection are of sufficient enough quality. The cells which express the antigen targeted by the ferrofluid will align along the ferromagnetic lines in addition to the cells which nonspecifically bound the ferrofluid, cells which were captured due to other reasons such as entrapment will not align and thus results in a further purification of infrequent cell types. Identification and further characterization of the target cells is obtained by the differences in the scattered and spectrum of the fluorescence light. An additional improvement can be achieved by utilization of a fluorescent form of the bioactive ferrofluid, the target cells can then be discriminated from non specifically bound cells by the amount of fluorescence emitted by the cells, i.e. the density of the antigen on the cell surface of the target cell is most likely different from the density of nonspecifically bound ferrofluid to non target cells. In contrast with flowcytometry the individual targets can be reexamined in that the optical detection system can identify and locate the target particles of interest, the receiver then can be examined by a more sophisticated optical detection system as such as a confocal microscope.

The terms and expressions which have been employed are used as terms of description and not of limitation and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described, or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed.

WHAT IS CLAIMED IS:

1. A method for quantitatively analyzing the particulate content of a test sample comprising particles of a target substance present in a fluid carrier, said particles having selectable response characteristics to impingement thereon of focused rays of radiant energy in the optical wave band, said method comprising the steps of:

providing a receiver having a wall portion that permits transmission of said radiant energy, said receiver including capture means for immobilizing said particles in a linear array, said capture means operable to receive and immobilize particles from a zone in said receiver having a predetermined volume;

introducing a quantity of said test sample into said receiver for a time sufficient to allow said particles to be immobilized on said capture means in a substantially linear array corresponding in thickness to about the size of said particles;

directing focused rays of radiant energy in the optical range along said linear array of particles; and

detecting the response of said particles within said zone to the impingement of said radiant energy thereon as a measure of the quantity of said particles in said test sample.

2. A method as claimed in claim 1, wherein said wall portion of said receiver has a surface with said capture means superimposed thereon and said detecting step comprises detecting said response over an area of said wall surface including at least a portion of said linear array of particles, as measure of the proportion of said detected particles in said

predetermined volume.

3. A method as claimed in claim 2, wherein said particles are bioparticles magnetically labelled with colloidal magnetic particles, and said capture means comprises a magnetic line which is magnetized to generate a magnetic field gradient within said receiver along said magnetic line, thereby to cause immobilization of said magnetically labelled bioparticles on said magnetic line.

4. A method as claimed in claim 2, wherein said particles are non-magnetic bioparticles, said bioparticles comprising or being adapted to comprise at least one binding site, said capture means comprising a line of binding agent having specific binding affinity for said at least one binding site of said bioparticles and said suspension in said receiver is subjected to conditions causing binding of said bioparticles to said binding agent.

5. A method as claimed in claim 1, wherein said test sample comprises whole blood.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant or Patentee: Leon W.M.M. Terstappen

Provisional Application No.: Not Yet Assigned

Filed or Issued: Concurrently Herewith

For: APPARATUS AND METHOD FOR CELL ANALYSIS

VERIFIED STATEMENT (DECLARATION) SUPPORTING ANOTHER'S CLAIM FOR SMALL ENTITY STATUS [37 CFR §1.9(f) and §1.27(c)] - SMALL BUSINESS CONCERN

I hereby declare that I am making this verified statement to support a claim by the above-identified applicant or patentee for small entity status for purposes of paying reduced fees with regard to the above-identified invention described in

[X] the specification filed herewith
[ ] U.S. Patent Application No. _____, filed _____
[ ] U.S. Patent No. _____, issued _____

I hereby declare that I am empowered to act on behalf of the small business concern identified below:

[ ] I am the owner.
[X] I am empowered to act as President and CEO of the concern.

Full name of the concern: IMMUNICON CORPORATION
Address of the concern: 1310 Masons Mill Business Campus, Huntington Valley, PA 19006

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR §121.3-18, and reproduced in 37 CFR §1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the above-identified small business concern with regard to the above identified invention.

If the rights held by the small business concern are not exclusive, each individual, concern or organization known to have rights to the invention is listed below* and the concern knows of no rights to the invention being held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR §1.9(c) if that person had made the invention, or by any concern which would not qualify as a small business concern under 37 CFR §1.9(d) or by a nonprofit organization under 37 CFR §1.9(e).

FULL NAME:
ADDRESS:

[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION
FULL NAME:
ADDRESS:
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION
FULL NAME:
ADDRESS:
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR §1.27)

I acknowledge the duty to file, in this application or patent, notification of any changes in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR §1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing: Paul A. Liberti

Title in Organization : President and CEO

Address : 1310 Masons Mill Business Campus, Huntingdon Valley, PA 19006

Signature _____ **Date:** Jan 7, 1996

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant or Patentee: Leon W.M.M. Terstappen

Provisional Application No.: Not yet Assigned

Filed or Issued: Concurrently Herewith

For: APPARATUS AND METHOD FOR CELL ANALYSIS

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR §1.9(f) AND §1.27(b)) - INDEPENDENT INVENTOR(S)

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR §1.9(c) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled described in

[X] the specification filed herewith
[ ] U.S. Patent Application No. _____, filed _____
[ ] U.S. Patent No. _____, filed _____

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR §1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR §1.9(d) or a nonprofit organization under 37 CFR §1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

[ ] no such person, concern, or organization
[X] person, concerns or organizations listed below*

FULL NAME: IMMUNICON CORPORATION
ADDRESS: 1310 Masons Mill Business Campus, Huntingdon Valley, PA 19006

[ ] INDIVIDUAL [X] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION
FULL NAME:
ADDRESS:

[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION
FULL NAME:
ADDRESS:

[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Leon W.M.M. Terstappen
Name of Inventor

Signature of Inventor

Date June 7, 1996

Name of Inventor

Signature of Inventor

Date

Name of Inventor

Signature of Inventor

Date

Name of Inventor

Signature of Inventor

Date

FIG 1A

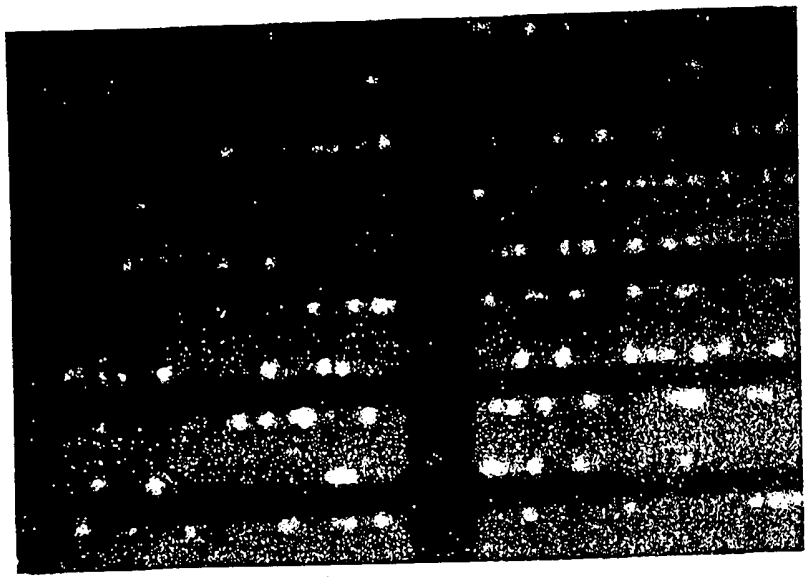
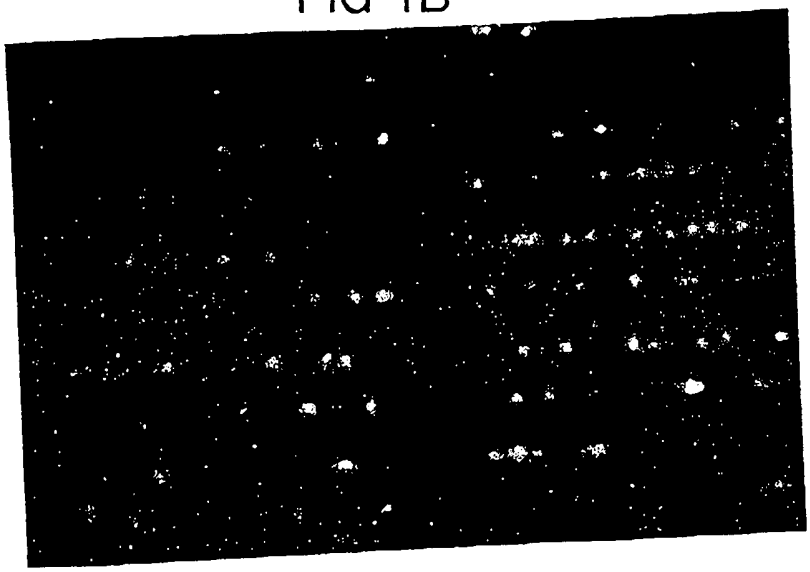


FIG 1B



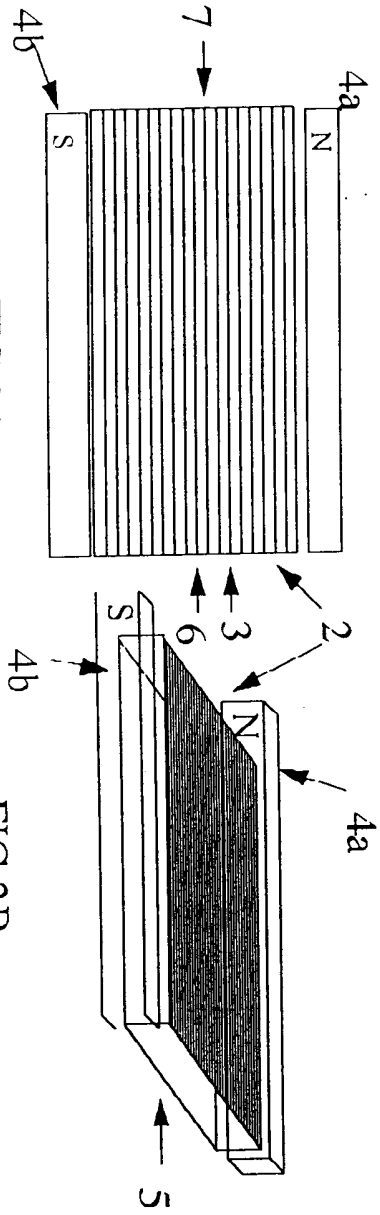


FIG 2A

FIG 2B

PTO/SB/68 (11-96)
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Under the Paperwork Production Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS OF ABANDONED APPLICATION UNDER 37 CFR 1.14(A)

	In re Application of **Dolan et al**	
	Application Number **60/019,282**	Filed **6/7/96**
	Group Art Unit	Examiner
Assistant Commissioner for Patients Washington, DC 20231		**Paper No.** 2

I hereby request access under 37 CFR 1.14(a)(3)(iv) to the application file record of the above identified ABANDONED application, which is: (CHECK ONE)

☒ (A) referred to in United States Patent Number **5,985,153**, column _____.

☐ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11, i.e., Application No. _____, filed _____, on page _____ of paper number _____.

☐ (C) an application that claims the benefit of the filing date of an application that is open to public inspection, i.e., Application No. _____, filed _____, or

☐ (D) an application in which the applicant has filed an authorization to lay open the complete application to the public.

Please direct any correspondence concerning this request to the following address:

Barbara J. Hendley
Signature

Barbara J. Hendley
Type or Printed Name

6/1/00
Date

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